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Hybridization of Non-Sulfonylurea Insulin Secretagogue and Thiazolidinedione-Derived Insulin Sensitizer

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Abstract—Hybrid compounds of non-sulfonylurea insulinotropic agents and thiazolidinedione-derived insulin-sensitizing agents were designed and synthesized. The benzylidenesuccinic acid derivative **24** was equal both to nateglinide in potency of insulin-releasing activity and to pioglitazone in insulin-sensitizing activity.

Introduction

Type 2 diabetes (non-insulin-dependent diabetes mellitus) is a progressive metabolic disorder characterized by defective insulin secretion from pancreatic β-cells and insulin resistance in peripheral tissues. Accordingly, both enhancement of insulin secretion and reduction of insulin resistance may be of importance for its treatment. Combination therapy of sulfonylurea and insulin sensitizers has been reported to exert an additional antihyperglycemic effect compared with sulfonylurea alone.[1] Therefore, we considered that a compound possessing both insulinotropic and insulin-sensitizing activity would be promising anti-diabetic agent.

The recently developed non-sulfonylurea class of insulinotropic agents includes two, nateglinide[2] and KAD-1229,[3] which have a β-phenylpropionic acid structure with amide functionality adjacent to the carboxyl group. Meanwhile, a series of 5-benzylthiazolidine-2,4-dione (TZD) compounds (e.g. troglitazone and pioglitazone)[4] and analogous phenylpropionic acid derivatives[5] have been developed as insulin-sensitizing agents. Of these, the amide-containing β-phenylpropionic acid **A**—the main metabolite of the isoxazolidinedione insulin sensitizer JTT-501—has been reported as showing potent insulin-sensitizing activity.[6] Intrigued by the structural similarity between the non-sulfonylurea insulin secretagogues and the insulin sensitizers, we designed hybrid molecules of these distinct hypoglycemic agents based on a β-phenylpropionic acid (Chart 1).

Chemistry

Pioglitazone was selected as the prototype from among a number of TZD insulin sensitizers as it has been reported to have moderate insulinotropic activity.[7] Scheme 1 shows the preparation of the nateglinide–pioglitazone hybrid molecule. The *N*-acylated tyrosine **1**, which was prepared by condensation of DL-tyrosine methyl ester with *trans*-4-isopropylcyclohexanecarboxylic acid, was treated with 2-(5-ethyl-2-pyridyl)ethanol under Mitsunobu reaction conditions. Saponification with 2 N NaOH gave the desired hybrid molecule **2**. The KAD-1229–pioglitazone hybrid molecule (**9**, R = 5-ethyl-2-pyridyl) and related compounds were prepared as shown in Scheme 2. The (*E*)-benzylidenesuccinate **5** was obtained by Stobe condensation between 4-hydroxybenzaldehyde (**3**) and diethyl succinate (**4**) in the presence of 2.5 equiv of NaOEt. Catalytic hydrogenation of **5** followed by coupling with *cis*-hexahydroisoindoline gave the amidoester **7**. *O*-Alkylation of **7** with the tosylate of 2-(5-ethyl-2-pyridyl)ethanol and subsequent saponification afforded the desired hybrid **9** as a racemate. The α,β-unsaturated analogue **10** was obtained by omitting the above catalytic hydrogenation, or alternatively from the known *O*-alkylated benzaldehyde **11**.[8] The optically pure (*S*)-isomer of **9** was prepared from 2-(4-benzyloxy) phenylpropionic acid modified with Evans' chiral auxiliary[9] (*R*)-4-benzyl-1,3-oxazolidine-2-one utilizing highly diastereoselective alkylation of bromoacetate as shown in Scheme 3. (*R*)-**9** was obtained by using an enantiomer of the auxiliary.[10]

Results and Discussion

Compounds were screened for insulin-releasing activity in HIT-T15 cells in the presence of 16.7 mM of

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glucose[11] and for insulin-sensitizing activity by measuring the triglyceride accumulation resulting from insulin-regulated differentiation of 3T3-L1 cells.[12] Values in Table 1 are given as a percentage of nateglinide response for insulin-releasing activity and of pioglitazone response for insulin-sensitizing activity.

As indicated in Table 1, simple hybridization was found to result in decrease of both activities. Although (*S*)-**9** showed almost the same insulinotropic activity as nateglinide at a concentration of 10 μM, little insulin-sensitizing activity was observed. It has been reported that L-tyrosine-based insulin sensitizers have more potent activity

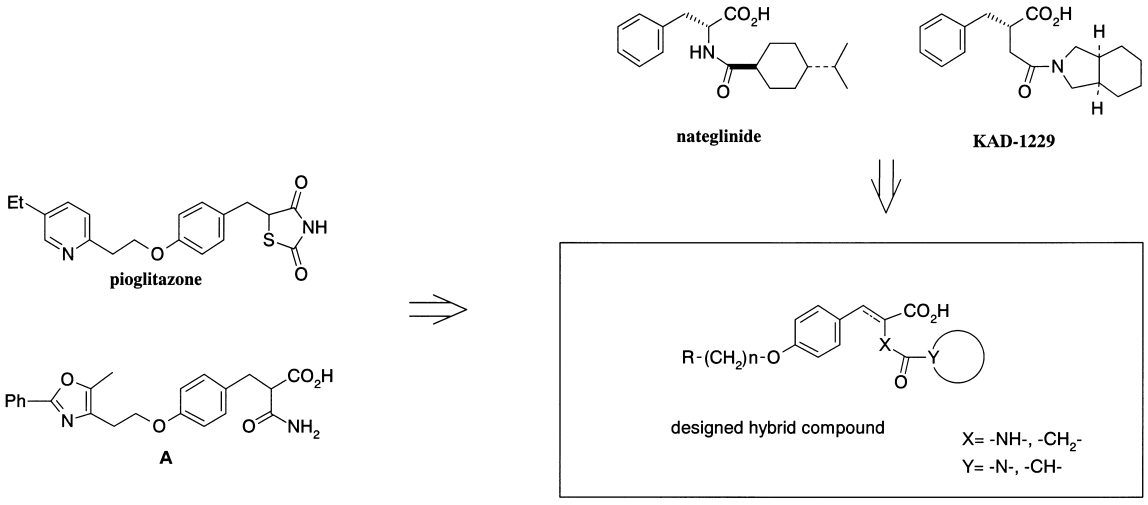
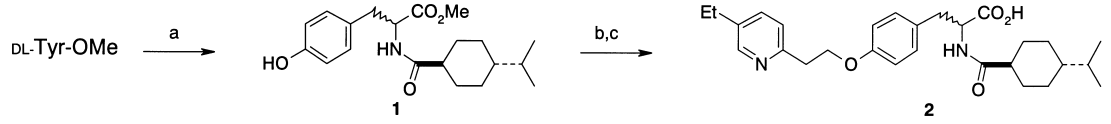
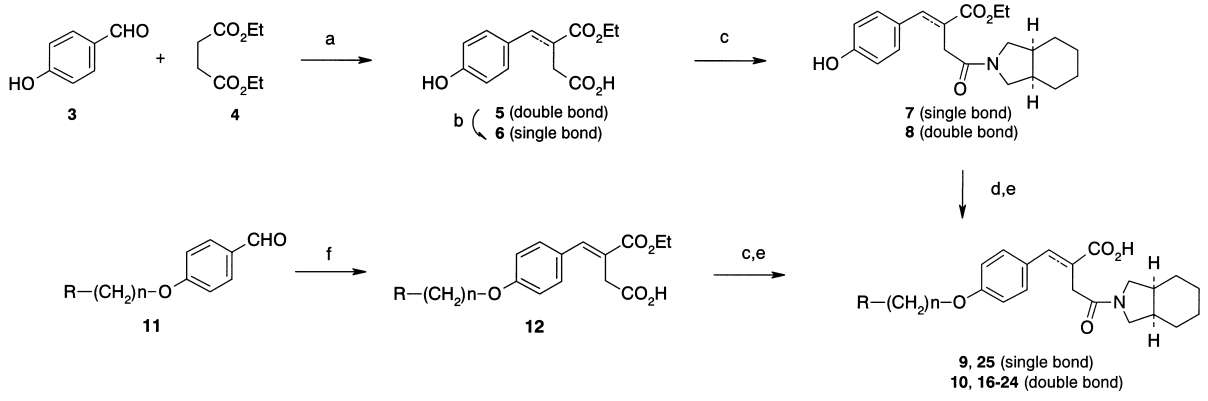


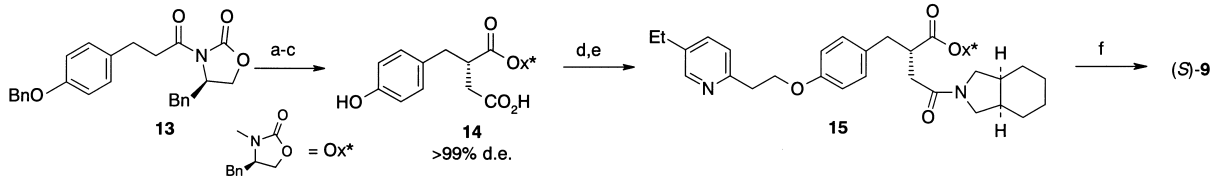
Chart 1.



Scheme 1. Synthesis of nateglinide–pioglitazone hybrid molecule. Reagents: (a) *trans*-4-isopropylcyclohexanecarboxylic acid, WSCD-HOBt; 78%; (b) 2-(ethyl-2-pyridyl)ethanol, Ph_3P, DEAD, THF, 46%; (c) 2 N NaOH, 81%.



Scheme 2. Synthesis of KAD-1229-pioglitazone hybrid molecule and related compounds. Reagents: (a) NaOEt (2.5 equiv), EtOH, reflux 72%; (b) H_2–Pd/C, EtOH, 99%; (c) *cis*-hexahydroisoindoline, WSCD-HOBt, DMF; (d) tosylate of R-$(CH_2)_n$-OH, K_2CO_3, CH_3CN, reflux; (e) 2 N NaOH; (f) NaOEt (1.5 equiv), EtOH, reflux.



Scheme 3. Synthesis of chiral KAD-1229-pioglitazone hybrid molecule. Reagents: (a) LDA, $BrCH_2CO_2Bu^t$, THF and then crystallization, 58%; (b) H_2–Pd/C; (c) TFA, 67% (two steps); (d) *cis*-hexahydroisoindoline, Bop reagent, DMF, 62%; (e) 2-(5-ethyl-2-pyridyl)ethanol, Ph_3P, DEAD, THF, 24%; (f) LiOOH, THF, 95%.

than their D-antipodes,[5e] but also that the L-antipode of nateglinide shows little correlation with insulinotropic activity.[13] This finding suggested that our target hybrid molecules ought to be achiral ones. Encouragingly, the benzylidenesuccinic acid compound **10** showed increase in both activities compared to the saturated racemic **9**. We therefore next synthesized the benzylidenesuccinic acids **16**–**24**, in which the pyridyl moiety of **10** was substituted with other aryl- or arylamino groups found in TZD-based insulin sensitizers. Synthesis of these analogues was carried out in a similar manner to that illustrated in Scheme 2.

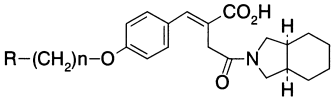
Table 1. Biological activities of hybrid molecules (R = 5-Et-2-pyridyl)

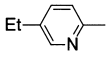
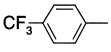
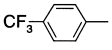
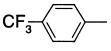
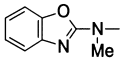
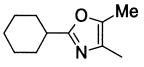
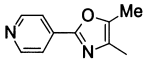
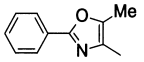
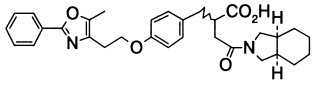
Compound	Insulin-releasing[a] activity at 10 μM	Triglyceride accumulation[b] (insulin-sensitizing activity)	
		at 10 μM	at 1 μM
2	33	30	2
9	68	30	8
(*S*)-**9**	105	11	4
(*R*)-**9**	1	*55*	17
10	136	50	20
Nateglinide	100	40	6
Pioglitazone	10	100	100

[a]% activity of nateglinide. Values are means of four experiments.
[b]**%** activity of pioglitazone. Values are means of three experiments.

Table 2 summarizes screening data for the synthesized benzylidenesuccinic acid hybrid molecules. Replacement of the pyridyl group of **10** with 4-substituted phenyl or 1-indolyl groups to give **16**–**19** decreased insulin-sensitizing activity, although insulin-releasing activity was maintained. In contrast, compounds **20**–**21**, which possessed *N*-methylheteroaryl groups derived from BRL-49653 and its analogues,[14] showed increased insulin-sensitizing activity but with reduced insulinotropic activity. Substitution with the 5-methyl-2-phenyloxazolyl group to give **24** successfully increased both activities. We also prepared compound **25** as a saturated analogue of **24**. Again, the potency of activity was reduced. This result corroborates the importance of the α,β-unsaturated structure of benzylidenesuccinic acid for both activities of the present hybrid molecules.

Table 2. Screening data for benzylidenesuccinic acid hybrid molecules[a]



Compound	R	*n*	Insulin-relasing activity at 10 μM	Triglyceride accumulation at 1 μM
10		2	136	20
16		1	130	15
17		2	93	5
18		3	130	4
19		2	105	9
20		2	60	87
21		2	60	121
22		2	83	93
23		2	49	93
24		2	145	103
25			85	45
Nateglinide			100	6
Pioglitazone			10	100

[a]See footnotes to Table 1.

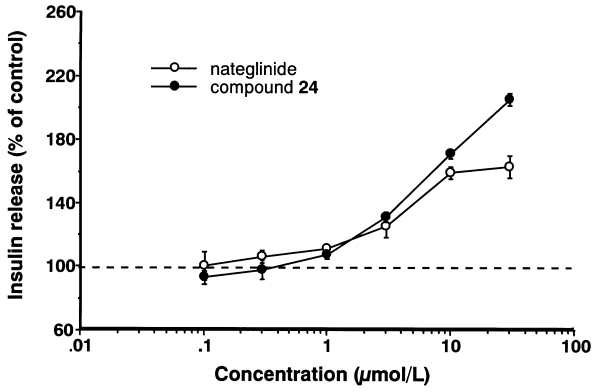


Figure 1. Effects of compound **24** and nateglinide on insulin release in HIT-T15 cells. Values represent the mean±S.E.M. ($n=4$).

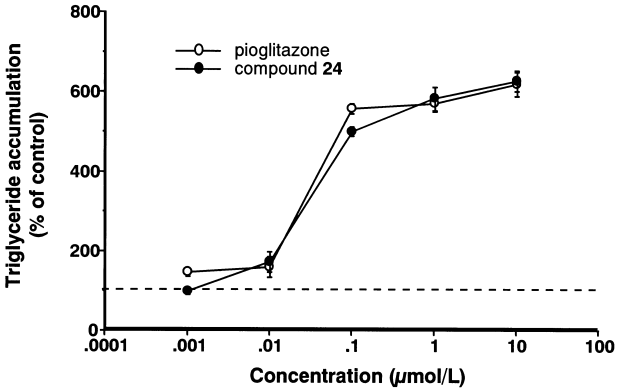


Figure 2. Effects of compound **24** and pioglitazone on triglyceride accumulation in 3T3-L1 cells. Values represent mean± S.E.M. ($n=3$).

For compound **24**, dose-dependency of the activities was examined. Compound **24** stimulated insulin secretion significantly from concentrations of 3×10^{-6} M and potency was almost the same as that of nateglinide. It was noteworthy that it displayed greater efficacy than nateglinide at concentrations of 10 μM or more (Fig. 1). Compound **24** also exhibited a similar triglyceride-accumulation profile to pioglitazone in 3T3-L1 cells (Fig. 2).

In summary, we designed and synthesized hybrid molecules of non-sulfonylurea insulin secretagogues and TZD-derived insulin sensitizers on the basis of their common structure. We demonstrated that a benzylidenesuccinic acid structure plays an important role in enhancing both insulin-secreting and -sensitizing activities. In particular, a hybrid molecule with a phenyloxazolyl group (compound **24**)[15] exhibited potency in both insulin-related activities. To the best of our knowledge, this is the first example of a potent insulin-releasing and -sensitizing dual agent. Study of the present compounds in vivo and further optimization are in progress.

References and Notes

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10. Optical purity was determined to be >99% e.e. by HPLC using a Daicel CHIRALCEL® AD column. Specific rotations, $[\alpha]_D^{25}$ ($c=0.50$, $CHCl_3$), were +25.2° for (*S*)-**9** and 25.8° for (*R*)-**9**.
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15. Data of **24**: colorless crystals, mp 118–120 °C, ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 1.30–1.69 (8H, m), 2.14–2.33 (2H, m), 2.38 (3H, s), 2.99 (2H, t, $J=6.6$ Hz), 3.25–3.53 (6H, m), 4.26 (2H, t, $J=6.6$ Hz), 6.90 (2H, d, $J=8.6$ Hz), 7.29 (2H, d, $J=8.6$ Hz), 7.36–7.48 (3H, m), 7.82 (1H, s), 7.93–8.01 (2H, m). FABMS (*m*/*z*) 515.